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Original Article

Variation of Phenolic Acid Compounds in the Iranian Germplasm of Boraginaceae, a Chemotaxonomy Approach

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ABSTRACT

In this study, phenolic acid compositions in fourteen taxa of Boraginaceae were analyzed by HPLC-UV to obtain informative chromatographic data. In general, 9 phenolic acid compounds were identified in different studied taxa. m-coumaric acid (1.80-1962.09 mg/kg), salicylic acid (13.22-867.35 mg/kg), ferulic acid (0.00-661.69 mg/kg), and p-coumaric acid (12.10-392.48 mg/kg) were characterized as the main compounds of the studied taxa. The results indicated that m-coumaric acid was the main phenolic acid in *Heterocaryum rigidium*, *Myosotis sylvatica*, and *Solenanthus stamineus*. Furthermore, salicylic acid was the main phenolic acids of *Myosotis sylvatica* and *Nonnea caspica*. Also, the highest amounts of ferulic acid and p-coumaric acid were detected in *Myosotis sylvatica* and *Heliotropium europaeum*, respectively. The qualitative and quantitative evaluation of these compounds was used for the characterization of the taxa and for revealing their phytochemical similarity and differentiation. Hierarchical cluster analysis showed that the studied taxa were classified into two main clusters (I and II) based on main phenolic acid compounds. Cluster I included *Heterocaryum rigidum*, *Solenanthu stamineus*, and *Lappula microcarpaa*, and the rest studied taxa grouped in cluster II. The chemotaxonomic significance of the isolated compounds was discussed.

INTRODUCTION

Natural product researches are of interest due to their pharmacologically activity and chemotaxonomic approaches in systematic studies. Phytochemical studies provide a useful tool for the comparison of medicinal species with species that have been unrecognized their medicinal properties. Also, phytochemical studies of unrecognized species may provide knowledge about new sources of natural bioactive compounds. On the other hand, in recent years, chemometric approaches, based on analytical data to characterize and classify taxa at different taxonomic ranks, have been proposed [1-3]. The alkaloids, fatty acids, terpenoids, and phenolic compounds are the main groups of compounds used for chemotaxonomic classification [4].

Boraginaceae Juss. is one of the largest and most important families of eudicots plants. It consists of around 146 genera and 2000 species with many economically important herbs, distributed throughout the tropical, subtropical, and temperate regions of the world [5]. The family comprises a group of plants that are important for pharmacology and cosmetology. The therapeutic effect of these plants is related to the content of many biologically active compounds, such as phenolic compounds.

Phenolic compounds are the main class of secondary metabolites in plants and are divided into phenolic acids, flavonoids, coumarins, lignins, and tannins. These compounds play a main role in plants by adjusting their growth as an internal physiological regulator. Among these classes, phenolic acids, flavonoids, and tannins are considered the main dietary phenolic compounds [6,7]. Chemically, phenolic acids have at least one aromatic ring where at least one hydrogen is substituted by a hydroxyl group and categorized into two groups including hydroxybenzoic acids and hydroxycinnamic acids [8]. These compounds are produced by the shikimate pathway, in which L-phenylalanine or L-tyrosine is the precursory substance [9]. Phenolic acids are often included in the human diet and have been largely

studied due to their bioactivities, such as antioxidant, anti-cancer, anti-viral, antimicrobial, anti-inflammatory, and anti-allergic. They are also used in the cosmetic and food industries as natural antioxidants [10-13].

There are several studies about the significance of phenolic compounds as chemical markers in botanical chemosystematic studies. Dresler *et al*. [14] studied some phenolic metabolite content of Boraginaceae species and concluded that the presence and abundance of allantoin, p-hydroxybenzoic acid, rutin, hydrocaffeic acid, rosmarinic acid, and chlorogenic acid could be used for the characterization of the species and for revealing their phytochemical similarity and differentiation. Lemma *et al*. [2] showed that the relative abundance of benzoic and coumaryl derivatives can be used as a proxy to distinguish *Erica* species from other plants. Watanabe *et al*. [15] identified four phenolic compounds including trans-cinnamic acid, lactic acid, rutin, and protocatechuic acid and concluded these compounds are necessary for chemotaxonomy.

Based on our knowledge, there is no study on phenolic compounds of Iranian taxa of Boraginaceae family. So, this study aimed to determine phenolic acid compounds diversity and the phytochemical relationship among some species of Boraginaceae family and evaluation of their significance as chemical markers for taxonomic purposes.

MATERIAL and METHODS

Plant Material

The aerial parts of the investigated taxa were collected from different natural habitats in Iran (Table 1) and voucher specimens were deposited in the Herbarium of Azarbaijan Shahid Madani University (ASMUH). Specimens were identified by Dr. Nikzat according to *Flora Iranica* [16].

Chemicals

Solvents and chemicals used for the extraction were purchased from Merck, German. All phenolic acid standards (named Gallic acid, p-Hydroxybenzoic acid, Vanillic acid, Caffeic acid, p-Coumaric acid, Ferulic acid, Salicylic acid, m-Coumaric acid, Cinnamic acid) were developed from Sigma Aldrich, which was > 95% pure. Also, the solvents used in the HPLC were grade HPLC.

Extraction of Phenolic Acids

The extraction of phenolic acids was carried out according to the method described by Hazrati *et al*. [17] with some modification. Briefly, 10 mL of 80% ethanol was added to 1.0 g of the dried powdered plant, and then vigorously shaken. After centrifuging at speed of 6000 × g for 5 min, the supernatant was collected, evaporated, and then stored in an amber glass vial (-20 °C in a freezer) until analysis by HPLC.

Analysis of Phenolic Acids

The analysis of phenolic compounds was done by the use of an HPLC (Waters 2695, USA) system equipped with a diode-array detector, a 20 µl loop, and an ODS column (250 mm × 0.46 mm, 5 µm). The reverse-phase separation was carried out with gradient elution solvent A and B being methanol-TFA (99.9:0.1, v/v) and water-TFA (99.9:0.1, v/v), respectively. Gradient conditions were: 20% A, in 0 min; 30% A, in 10 min; 60% A, in 30 min; 80% A, in 40 min; 100% A, in 45 min; 20% A, in 52 min; isocratic, 6 min. The flow rate of the mobile phase was adjusted at 1 mL/ min and the wavelength was maintained at 254, 275, and 320 nm [18]. Quantification of the phenolic acids was based on multilevel external calibration curves with a linear range over 0.1–20 µg/mL for gallic acid; 0.2–50 µg/mL for p-hydroxybenzoic acid, vanillic acid, caffeic acid, p-coumaric acid, and cinnamic acid; and 0.2–200 µg/mL for ferulic acid, salicylic acid, and m-coumaric acid.

Statistical Analysis

In this study, each experiment was performed with at least three replications. For statistical analysis, the data were subjected to statistical software of PAST (ver. 2.17). A principal components analysis (PCA-biplot) was used to recognize the most variable phenolic compounds between the studied taxa. In order to determine chemical distance among studied taxa, correspondence analysis (CA) and hierarchical cluster analysis (HCA) were created via the ward's method.

Table 1 List of studied taxa names, locality, and voucher numbers.

Taxon	Locality	Voucher No.
Anchusa italica Retz.	Tehran, Damavand	ASMUH99001
Arnebia decumbens (Vent.) Coss. & Kralik	Tehran	ASMUH99002
Asperugo procumbens L.	Tehran, Damavand	ASMUH99003
Cynoglossum officinale L.	Tehran, Damavand	ASMUH99004
Echium italicum L.	Tehran, Rudehen	ASMUH99005
Heliotropium europaeum L.	Mazandaran, Sari	ASMUH99006
Heterocaryum rigidum A.DC.	Tehran, Jajrod	ASMUH99007
Lappula microcarpa (Ledeb.) Gürke	Tehran, Damavand	ASMUH99008
Myosotis sylvatica Hoffm.	Mazandaran, Pol-Sefid	ASMUH99009
Nonnea caspica G. Don	Tehran	ASMUH99010
Onosma microcarpum DC.	Tehran, Damavand	ASMUH99011
Rochelia persica Bunge ex Boiss.	Mazandaran, Chalus	ASMUH99012
Solenanthus stamineus (Desf.) Wettst.	Tehran, Damavand	ASMUH99013
Symphytum asperrimum Donn ex Sims	Mazandaran, Qaemshahr	ASMUH99014

RESULTS

In this study, the amounts of phenolic acids were quantified at 14 species of the Boraginaceae family in Iran (Table 2). In general, 9 phenolic acid compounds were evaluated in different studied species using HPLC-UV. The amount of phenolic acids is expressed as mg/kg dried weight (mg/kg DW), and based on the results (Fig. 1), the highest amounts were associated with *L. microcarpa* (2424.9 mg/kg DW), *M. sylvatica* (2247.9 mg/kg DW), *H. rigidium* (2128.5 mg/kg DW), and S. solnathus (2038.1 mg/kg DW). *A. procumbens* and *E. italicum* had the lowest phenolic acids with values of 49.4 and 75.1 mg/kg DW, respectively.

According to the information of Table 2, p-coumaric acid, salicylic acid, and m-coumaric acid were present in all species, and their highest amount was correlated with *H. europaeum*, *M. sylvatica*, and *H. rigidium* with values of 392.48, 871.14, and 1962.07 mg/kg DW, respectively. The results showed that m-coumaric acid was the main phenolic acid in *H. rigidium*, *L. microcarpa*, *A. decumbens*, *N. caspica*, *S. stamineuss*, and *C. officinle*, and para-hydroxy benzoic acid was the main phenolic acid in *A. procumbens* and *O. microcaryum*. Furthermore, salicylic acid was the main phenolic acid of *M. sylvatica* and *A.italic*. Fig. 2 indicates the major phenolic in taxa which had the highest total phenolic acids. m-Coumaric acid was the main phenolic acid in *H. rigidium*, *S. stamineus*, and *L. microcarpa*, while in *M. sylvatica*, Salicylic acid was the main phenolic acid.

PCA-biplot was used to show the most significant phenolic compounds among the studied species (Fig. 3). The first PC1 explained 84.48 % of the variation and had a positive correlation with MCA. PC2 explained SA as positive correlation and accounted for 11.19 % of the variance (Table 3). Since PC1 and PC2 which together explained 95.67 % of total variance in related chemical characters among species, hence scatter plot of PC1 and PC2 were used to determine chemical distance.

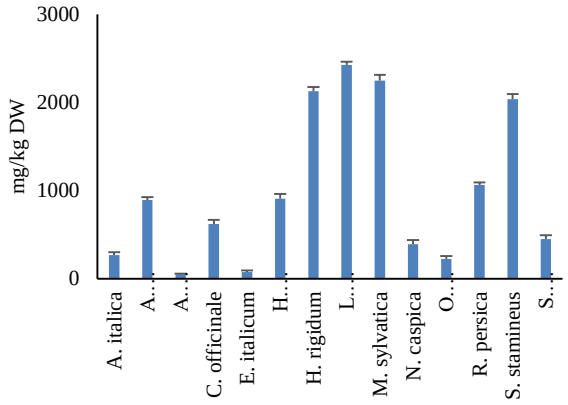

Fig. 1 Total amount of phenolic acid compounds (mg/kg dried weight) in some species of Boraginaceae

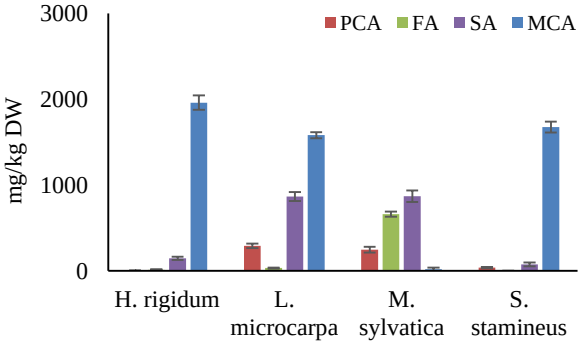

Fig. 2 Amounts of main phenolic acids in some taxa of Boraginaceae.

Table 2 Amount of phenolic acid compounds (mg/kg dried plant; mean ± S.E.) in some taxa of Boraginaceae family

Taxon	GA	PHBA	VA	CaA	PCA	FA	SA	MCA	CiA
A. italica	1.92±0.14	75.09±3.72	23.20±1.01	4.97±0.56	4.42±0.61	8.36±0.17	88.16±16.32	36.15±2.11	1.09±0.07
A. decumbent	0.00	18.49±1.12	16.28±0.62	2.88±0.06	52.02±1.94	98.76±3.03	54.96±1.87	617.84±27.54	31.74±1.24
A. procumbens	0.00	15.56±1.24	0.49±0.00	13.37±0.44	2.59±0.05	1.88±0.07	10.89±1.34	3.26±0.55	1.38±0.38
C. officinale	0.00	5.63±0.56	13.51±0.51	39.51±1.46	17.90±0.56	28.73±0.96	122.96±20.23	360.00±9.58	33.25±2.01
E. italicum	0.00	0.00	13.81±0.42	19.83±0.87	12.10±0.47	0.00	13.22±0.52	15.93±0.49	0.28±0.03
H. europaeum	0.00	171.66±7.22	32.03±1.16	11.86±0.48	392.48±14.32	76.71±2.16	214.76±7.81	8.17±0.13	1.57±0.18
H. rigidum	1.02±0.07	0.00	0.00	52.70±1.74	3.87±0.12	13.77± 0.46	147.27±5.25	1962.09±72.62	1.81±0.15
L. microcarpa	0.92±0.04	50.11±2.71	52.08±2.11	47.19±1.06	293.63±11.27	34.59±1.28	867.35±27.31	1582.39±35.63	0.98±0.09
M. sylvatica	2.57±0.21	71.32±3.00	84.92±2.85	241.13±9.65	247.84±8.95	661.69±22.17	871.14±31.27	19.49±0.57	47.64±2.78
N. caspica	2.11±0.27	17.53±0.82	18.00±0.83	0.00	95.30±3.21	13.56±0.50	40.32±0.84	175.78±5.23	30.50±2.12
O. microcarpum	0.00	157.05±7.29	4.65±0.13	8.29±0.31	17.98±0.69	2.87±0.06	32.57±0.91	1.80±0.29	0.00
R. persica	2.20±0.18	43.29±1.57	31.76±1.21	4.15±0.17	298.71±9.11	316.47±10.09	100.05±2.59	250.52±9.46	16.63±1.08
S. stamineus	0.00	56.37±1.37	36.62±1.56	181.17±6.94	37.74±1.51	0.00	75.81±2.61	1676.80±36.13	3.67±0.24
S. asperrimum	0.52±0.05	27.47±2.16	36.49±1.31	208.82±8.61	33.08±1.29	20.85±0.78	75.44±3.01	46.60±2.11	0.70±0.03

GA: Gallic acid; PHBA: p-Hydroxybenzoic acid; VA: Vanillic acid; CaA: Caffeic acid; PCA: p-Coumaric acid; FA: Ferulic acid; SA: Salicylic acid; MCA: m-Coumaric acid; CiA: Cinnamic acid

Table 3 Correlation of phenolic compounds of the studied taxa with two components of PCA.

Phenolic compounds	Component	
	1	2
PCA	-279.82	140.77
FA	-429.45	297.20
SA	96.73	827.59
MCA	2657.00	-145.35
GA	-460.85	-308.79
PHBA	-407.47	-198.30
VA	-413.55	-212.78
CaA	-314.00	-130.26
CiA	-448.57	-270.08

PCA: p-Coumaric acid; FA: Ferulic acid; SA: Salicylic acid; MCA: m-Coumaric acid; GA: Gallic acid; PHBA: p-Hydroxybenzoic acid; VA: Vanillic acid; CaA: Caffeic acid; CiA: Cinnamic acid

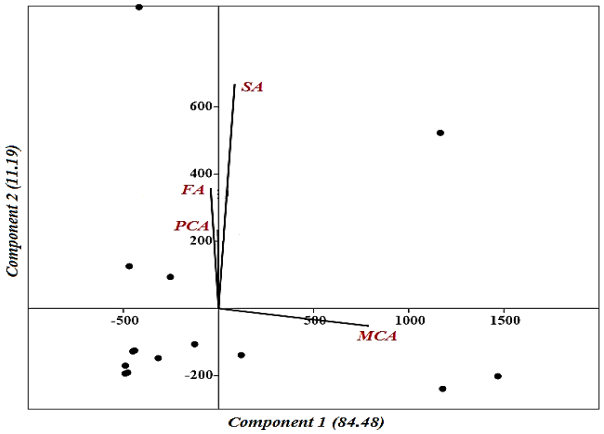

Fig. 3 Principal component analysis among studied taxa of Boragenaceae based on phenolic compositions.

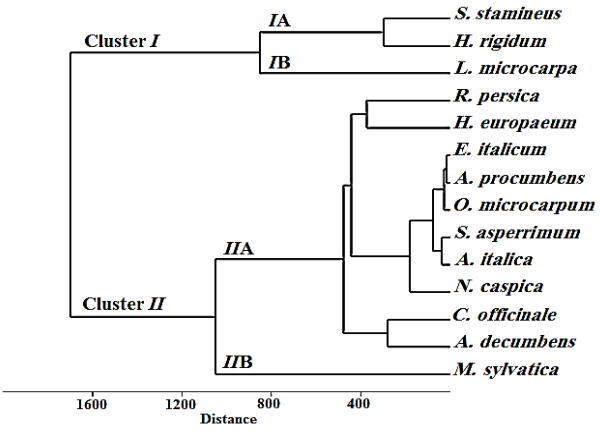

Fig. 4 Cluster analysis of studied taxa of Boraginaceae based on the phenolic compositions.

Hierarchical cluster analysis showed that the studied species were classified into two main clusters (I and II) based on phenolic compounds (Fig.4). Cluster I was divided into two sub-clusters sub-cluster *I*A included *H. rigidum* and *S. stamineus* while sub-cluster *II*B contained *L. microcarpaa*. In the cluster *II*, *M. sylvatica* placed in one sub-cluster (*II*B) and the rest studied species were grouped within the other sub-cluster (*II*A).

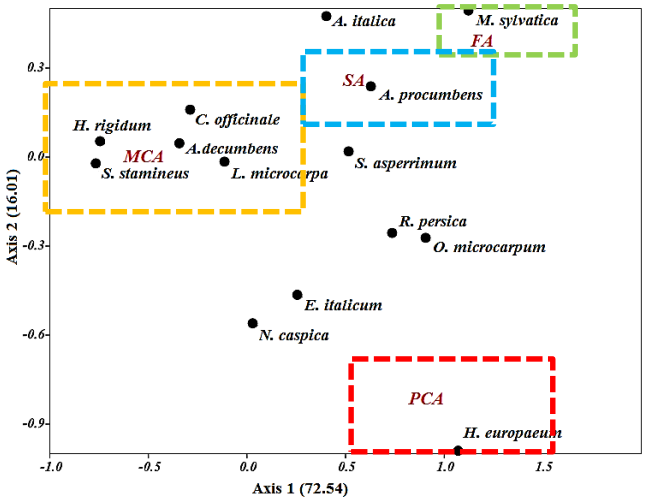

Fig. 5 Correspondence analysis of studied taxa of Boraginaceae based on the phenolic compositions.

In order to investigate the relationships among phenolic acid compounds of studies species, CA was used based on four major phenolic acid compounds (Fig. 5). Correspondence analysis showed two axes which together explained 70.47 % of total variance. The first and second axes (72.54 and 16.01% of variance) had significant positive correlations with fumaric acid and p-coumaric acid, respectively. According to CA plot, *H. rigidum*, *S. stamineus*, *L. microcarpa*, *C. officinale* and *A. decumbens* had meta-cumaric acid as the major compound While, fumaric acid was the main constituent in *M. sylvatica*. The other studied species had different amounts of phenolic compounds.

DISCUSSION

The knowledge that natural products provide a rich source for therapeutic discovery has led to the development of many of the world's most commonly used drugs. The chemical variation among the species should lead to the selection of plants with high potential in order to use them in the health care and food industry. Our results showed that the contents of MCA, PCA, FA, and CA in *H. rigidium*, *L. microcarpa*, *M. sylvatica*, and *S. stamineuss* are relatively high. These compounds are of particular interest because of their biological properties and potential applications in different industries. Several studies have reported that they can act as antioxidant, anti-inflammatory, antimutagenic, anti-ulcer, antiplatelet and anti-cancer activities, in addition to mitigating atherosclerosis, oxidative cardiac damage, UV-induced damage to ocular tissues, neuronal injury, anxiety, gout and diabetes [19, 20]. Park *et al*. [21] analyzed the aqueous and ethanolic extracts of roasted rice hulls and determined p-CA, VA, and FA as the dominant phenolic compounds. They showed that added roasted rice hull extracts, particularly rich in p-CA, protected against oxidative deterioration. Pieczykolan *et al*. [22] isolated salysilic acid-rich fractions from *Aerva lanata* (L.) Juss, and studied their antioxidant activity and ability to inhibit α-glucosidase and α-amylase. Bogucka-Kocka *et al*. [23] evaluated the phenolic acid compositions of *Kalanchoe* species and demonstrated that their extracts had significant cytotoxic and antioxidant effects. Thus, the previous studies and our results suggest the potential use of these plants as new sources of natural compounds in food and pharmaceutical industries.

The systematic classification of the Boraginaceae family has intrigued botanists for many years. The classification of this family has changed over time, especially since molecular data and phylogenetic analyses became available. Boraginaceae in the traditional sense [24] were subdivided into five subfamilies, namely Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, and Wellstedioideae. The recognition of natural subfamilies and tribes in the Boraginaceae has been challenged in many researches. Phylogenetic studies demonstrated that Boraginaceae in the traditional sense are paraphyletic [25-27] and provide new infrafamilial classification into tree subfamily and 11 tribes [28].

Evaluating the pattern of distribution of natural plant products is well established as a major tool for investigating accession structures, species, taxonomic problems and phyletic relationships among genera [29]. It is now possible to study phenolic profiles of low and high taxonomic levels [30].

In this study, the distributions of the phenolic compounds among studied species were varied and showed that phenolic profiles were valuable and can be used as a chemotaxonomic marker. The amount of MCA has the main role in the grouping of species in clades *I* and *II*. The results showed that an amount of MCA (>1000 mg/kg plant) was a distinct characteristic in the identification of *H. rigidium*, *S. stamineus* and *L. microcarpa* from the other taxa. A high amount of SA was the main characteristic of the recognition of *M. sylvatica*. There are many reports about significance of phenolic compounds as chemical markers in botanical chemosystematic studies [2, 30, 31]. Our results are in accordance with the finding of Dresler *et al*. [14], who used phenolic acid profiles for revealing phytochemical similarity and differentiation of Boraginaceae species.

On the other hand, the results of HCA demonstrated that phenolic compound profiles could not suitable for the investigation of evolutionary relationships. The distribution of the species was different from that was published by Chacon *et al*. [28] who studied phylogenetic relationships of the Boraginaceae. The phenolic compounds described here may be used in future studies aiming at completing the knowledge on Boraginaceae species.

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Authors' Contributions

Mstafa Ebadi and Amir Abbas Matin designed the study project, Bita Ghahraman, Saeed Mollaei and Sedighe Nikzat performed experiments and data analysis, and drafted the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

All authors declare no conflict of interest

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